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(54) Title: JUNCTIONAL ADHESION MOLECULE (JAM), A TRANSMEMBRANE PROTEIN OF TIGHT JUNCTIONS

(57) Abstract

A transmembrane protein located at tight junctions is disclosed. The protein is expressed in endothelial cells, epithelial cells, megakaryocytic cells and platelets. This protein is called junctional adhesion molecule (JAM). The amino-acid sequence of human JAM and of murine JAM are presented. In addition, the DNA sequences, the genes and recombinant proteins or peptides expressed by the genes or fragments of the genes are disclosed. Furthermore, antibodies binding specifically to JAM or a part of JAM and modifiers, i.e. inhibitors and inducers, of the polymerization of the transmembrane JAM are disclosed, together with the antibodies and the modifier as reagents in diagnostic kits, as well as applications of the modifier as vaccine adjuvant and as active ingredient in medicaments. Finally, transgenic animals or cells overexpressing or lacking JAM are comprised by the disclosure.

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JUNCTIONAL ADHESION MOLECULE (JAM), A TRANSMEMBRANE PROTEIN OF TIGHT JUNCTIONS

The present invention relates to a transmembrane component of tight junction, more precisely a new protein, which is expressed in endothelial cells, epithelial cells, megakaryocytic cells and platelets. This new protein is now denominated junctional adhesion molecule (JAM), and most of the human and the whole of the mouse JAM have been sequenced. The invention further comprises a cDNA coding for JAM, a structural gene coding for JAM, a recombinant protein or peptide expressed by the structural gene or by a fragment of the gene, an antibody specific for JAM, a modifier of the polymerization of transmembrane JAM, a diagnostic kit comprising the antibody or the modifier, a vaccine adjuvant comprising the modifier, a medicament comprising the modifier, and transgenic animals or cells overexpressing or lacking JAM. An example of the modifier is a monoclonal antibody specifically binding to JAM and preventing the polymerization of JAM at tight junctions, resulting inter alia in the blocking of leukocyte transmigration.

Background

The endothelium forms the main barrier to the passage of macromolecules and circulating cells from blood to tissues. Endothelial permeability is in large part regulated by intercellular junctions. These are complex structures formed by transmembrane adhesive molecules linked to a network of cytoplasmic/cytoskeletal proteins. At least four different types of endothelial junctions have been described: tight junctions, gap junctions, adherence junctions and syndesmos (Dejana et al., *infra*).

Intercellular tight junctions are responsible for the control of endothelial and epithelial cell layer permeability [Anderson et al; *Curr. Opin. Cell. Biol.* 5, 772-778 (1993)]. These organelles also regulate leukocyte transmigration, cell polarity and growth. The molecules which constitute the tight junctions are therefore good targets for developing drugs which affect inflammatory reaction, angiogenesis and cell proliferation in general [Dejana et al., *FASEB J.* 9, 910-918 (1995)].

Leukocyte transmigration through the endothelium or the epithelium in inflammation is associated with edema and tissue damage. To transmigrate leukocytes have to open the tight junctions and go through cell-cell contacts [Carlos and Harlan, Blood 84, 2068-2101 (1994)]. Tools which are able to specifically limit this process have 5 not been disclosed in the prior art.

Tumor cells seem to use similar mechanisms to transmigrate through the endothelium and infiltrate tissues. Agents which could prevent tight junction opening would be therefore useful in limiting tumor metastasis.

10 In addition, tight junctions are particularly important in the brain microvasculature where they are responsible for a tight control of permeability between the blood and the central nervous system [Risau and Wolburg, Trends Neurosci. 13, 174-178 (1990)]. This endothelial barrier constitutes a strong obstacle to the access of useful 15 drugs to the brain as for instance the penetration of chemotherapeutics for the cure of cerebral tumors. Methods to open or to repair the blood brain barrier without induction of endothelial damage have not yet been described in the literature.

20 Finally, the tight junctions are poorly expressed in epithelial cell derived tumors, and some tight junction components have oncosuppressor activity, i.e., their presence reduces the capacity of the tumor to proliferate and to metastasize [Tsukita et al., J. Cell Biol. 123, 1049-1053 (1993)]. Transfection of the tight junction molecule genes could be seen as a way to limit tumor progression. In addition, since tight junctions are needed for a correct organization of new vessels, inhibition 25 of their organization might prevent angiogenesis and thus inhibit the development of proliferative diseases such as cancer.

30 The molecular organization of tight junctions is so far only partially characterized. Only one transmembrane protein which is specific for tight junctions has been identified and denominated occludin [Furuse et al., J. Cell Biol. 123, 1777-1788 (1993)]. This protein connects specific cytoskeletal proteins inside the cells. The adhesive properties of occludin and its capacity to promote homotypic cell-to-cell interaction are not conclusively proven yet.

Description of the invention

The present invention is based on the finding of a new transmembrane protein located at tight junction. This new protein is called junctional adhesion molecule and is abbreviated JAM. This JAM protein, located at tight junctions, promotes cell-to-cell homotypic adhesion. The extracellular parts of the proteins adhere to each other on the same and adjacent cells by protein dimerization, oligomerization or polymerization in a zipper-like fashion and causes a strong reduction in paracellular permeability.

10

The JAM used in the experimental part of this specification is of mouse origin, whereas the JAM of human origin is the one which will be predominantly used as a model for the development of diagnostics and medicaments for use in, e.g., tumor therapy, angiogenesis control, control of Blood Brain Barrier, control of inflammatory response, control of transmigration of leukocytes, control of transmigration of other natural or engineered cells such as gene therapy in the brain.

Thus, the first aspect of the invention is directed to a protein in glycosylated or unglycosylated form comprising an amino-acid sequence selected from the sequence SEQ ID NO:1

(human)

Met Gly Thr Lys Ala Gln Val Glu Arg Lys Leu Leu Cys Leu Phe Ile
1 5 10 15

25

Leu Ala Ile Leu Leu Cys Ser Leu Ala Leu Gly Ser Val Thr Val His
20 25 30

Ser Ser Glu Pro Glu Val Arg Ile Pro Glu Asn Asn Pro Val Lys Leu
35 40 45

30

Ser Cys Ala Tyr Ser Gly Phe Ser Ser Pro Arg Val Glu Trp Lys Phe
50 55 60

35

Asp Gln Gly Asp Thr Thr Arg Leu Val Cys Tyr Asn Asn Lys Ile Thr
65 70 75 80

Ala Ser Tyr Glu Asp Arg Val Thr Phe Leu Pro Thr Gly Ile Thr Phe
85 90 95

40

Lys Ser Val Thr Arg Glu Asp Thr Gly Thr Tyr Thr Cys Met Val Ser
100 105 110

- 4 -

	Glu	Glu	Gly	Gly	Asn	Ser	Tyr	Gly	Glu	Val	Lys	Val	Lys	Leu	Ile	Val
	115								120					125		
5	Leu	Val	Pro	Pro	Ser	Lys	Pro	Thr	Val	Asn	Ile	Pro	Pro	Ser	Lys	Pro
	130								135					140		
	Thr	Val	Asn	Ile	Pro	Ser	Ser	Ala	Thr	Thr	Ile	Gly	Asn	Arg	Ala	Val
	145								150			155			160	
10	Leu	Thr	Cys	Ser	Glu	Gln	Asp	Gly	Ser	Pro	Pro	Ser	Glu	Tyr	Thr	Trp
													165		170	175
	Phe	Lys	Asp	Gly	Ile	Val	Met	Pro	Thr	Asn	Pro	Lys	Ser	Thr	Arg	Cys
15												180		185		190
	Leu	Gln	Gln	Leu	Phe	Leu	Ser	Ser	Leu	Asn	Pro	Thr	Thr	Gly	Leu	
												195		200		205
20	Val	Phe	Asp	Pro	Leu	Ser	Ala	Ser	Asp	Thr	Gly	Glu	Tyr	Ser	Cys	Glut
											210		215		220	
	Ala	Arg	Asn	Gly	Tyr	Gly	Thr	Pro	Met	Thr	Ser	Asn	Arg	Val	Ala	Met
	225								230			235			240	
25	Glu	Ala	Val	Asp	Gly	Asn	Val	Gly	Val	Ile	Val	Ala	Ala	Val	Leu	Val
											245		250		255	
	Thr	Leu	Ile	Leu	Leu	Gly	Ile	Leu	Val	Phe	Gly	Ile	Trp	Phe	Pro	Tyr
30									260		265			270		
	Ser	Arg	Gly	His	Phe	Asp	Arg	Thr	Lys	Lys	Gly	Thr	Ser	Ser	Lys	Lys
									275		280			285		
35	Val	Val	Tyr	Ser	Gln	Pro	Ser	Ala	Arg	Ser						
									290		295					

and homologous sequences having at least 72 % homology to the sequence SEQ ID NO: 1. The percentage of homology may for instance be 75%, 80% or as high as 85 %, or even higher, such as 90 % or 95 %, especially if the homologous sequence originates from a transmembrane protein of the same or closely related species. However, it is anticipated that proteins which have at least 72 % homology to this N-terminal sequence SEQ ID NO: 1 (some amino-acid residues at the C-terminal are missing from the full length protein) will share both diagnostic and medical properties to such a high degree that they can be used for the various applications of the present invention. Among such proteins may be included both

naturally occurring analogues and variants of the same or different species as well as synthetic or recombinant equivalents. An example of such a protein having at least 75 % homology to the SEQ ID: 1 is the mouse JAM protein having the amino-acid sequence SEQ ID NO: 2

5 (mouse)

	Met	Gly	Thr	Glu	Gly	Lys	Ala	Gly	Arg	Lys	Leu	Leu	Phe	Leu	Phe	Thr
1				5			10						15			

	Ser	Met	Ile	Leu	Gly	Ser	Leu	Val	Gln	Gly	Lys	Gly	Ser	Val	Tyr	Thr
10					20			25					30			

	Ala	Gln	Ser	Asp	Val	Gln	Val	Pro	Glu	Asn	Glu	Ser	Ile	Lys	Leu	Thr
15					35			40				45				

	Cys	Thr	Tyr	Ser	Gly	Phe	Ser	Ser	Pro	Arg	Val	Glu	Trp	Lys	Phe	Val
20					50			55			60					

	Gln	Gly	Ser	Thr	Thr	Ala	Leu	Val	Cys	Tyr	Asn	Ser	Gln	Ile	Thr	Ala
25					65			70			75			80		

	Pro	Tyr	Ala	Asp	Arg	Val	Thr	Phe	Ser	Ser	Ser	Gly	Ile	Thr	Phe	Ser
30					85				90				95			

	Ser	Val	Thr	Arg	Lys	Asp	Asn	Gly	Glu	Tyr	Thr	Cys	Met	Val	Ser	Glu
35					100			105				110				

	Glu	Gly	Gly	Gln	Asn	Tyr	Gly	Glu	Val	Ser	Ile	His	Leu	Thr	Val	Leu
40					115			120			125					

	Val	Pro	Pro	Ser	Lys	Pro	Thr	Ile	Ser	Val	Pro	Ser	Ser	Val	Thr	Ile
45					130			135			140					

	Gly	Asn	Arg	Ala	Val	Leu	Thr	Cys	Ser	Glu	His	Asp	Gly	Ser	Pro	Pro
50					145			150			155			160		

	Ser	Glu	Tyr	Ser	Trp	Phe	Lys	Asp	Gly	Ile	Ser	Met	Leu	Thr	Ala	Asp
55					165				170			175				

	Ala	Lys	Lys	Thr	Arg	Ala	Phe	Met	Asn	Ser	Ser	Phe	Thr	Ile	Asp	Pro
60					180			185				190				

	Lys	Ser	Gly	Asp	Leu	Ile	Phe	Asp	Pro	Val	Thr	Ala	Phe	Asp	Ser	Gly
65					195			200			205					

	Glu	Tyr	Tyr	Cys	Gln	Ala	Gln	Asn	Gly	Tyr	Gly	Thr	Ala	Met	Arg	Ser
70					210			215			220					

Glu Ala Ala His Met Asp Ala Val Glu Leu Asn Val Gly Gly Ile Val
 225 230 235 240

5 Ala Ala Val Leu Val Thr Leu Ile Leu Leu Gly Leu Leu Ile Phe Gly
 245 250 255

Val Trp Phe Ala Tyr Ser Arg Gly Tyr Phe Glu Thr Thr Lys Lys Gly
 260 265 270

10 Thr Ala Pro Gly Lys Lys Val Ile Tyr Ser Gln Pro Ser Thr Arg Ser
 275 280 285

Glu Gly Glu Phe Lys Gln Thr Ser Ser Phe Leu Val
 290 295 300 .

15 The synthetically or recombinantly produced proteins of the invention will function
 as competitors at tight junctions.

20 The second aspect of the invention is directed to a DNA sequence coding for a
 protein of the present invention, specifically a cDNA sequence coding for a protein
 of the present invention. Specific embodiments of this aspect of the invention are
 the cDNA sequence SEQ ID NO: 3
 (part of human)

25	TCCATTGTGC TCTAAAGCGG GACGCTGATC GCGATGGGA CAAAGGCGCA AGTCGAGAGG	60
	AAACTGTTGT GCCTCTTCAT ATTGGCGATC CTGTTGTGCT CCCTGGCATT GGGCAGTGTT	120
30	ACAGTGCACT CTTCTGAACC TGAAGTCAGA ATTCCTGAGA ATAATCCTGT GAAGTTGTCC	180
	TGTGCCTACT CGGGCTTTTC TTCTCCCCGT GTGGAGTGGGA AGTTTGACCA AGGAGACACC	240
	ACCAGACTCG TTTGCTATAA TAACAAGATC ACAGCTTCCT ATGAGGACCG GGTGACCTTC	300
35	TTGCCAACTG GTATCACCTT CAAGTCCGTG ACACGGGAAG ACACGGGAC ATACACTTGT	360
	ATGGTCTCTG AGGAAGGCGG CAACAGCTAT GGGGAGGTCA AGGTCAAGCT CATCGTGCTT	420
40	GTGCCTCCAT CCAAGCCTAC AGTTAACATC CCTCCATCCA AGCCTACAGT TAACATCCCC	480
	TCCTCTGCCA CCATTGGGAA CCGGGCAGTG CTGACATGCT CAGAACAAAGA TGGTTCCCCA	540
	CCTTCTGAAT ACACCTGGTT CAAAGATGGG ATAGTGTGATGC CTACGAATCC CAAAAGCACC	600
45	CGTTGCCTTC AGCAACTCTT CCTATCTAGT CTGAATCCCA CAACAGGAGA GCTGGTCTTT	660
	GATCCCCTGT CAGCCTCTGA TACTGGAGAA TACAGCTGTG AGGCACGGAA TGGGTATGGG	720
50	ACACCCATGA CTTCAAATCG TGTCGCGATG GAAGCTGTGG ACGGGAATGT GGGGGTCATC	780
	GTGGCAGCCG TCCTTGTAAC CCTGATTCTC CTGGGAATCT TGGTTTTGG CATCTGGTTT	840

CCGTATAGCC GAGGCCACTT TGACAGAAC AAGAAAGGG A CTTCGAGTAA GAAGGTAGTT	900
TACAGCCAGC CTAGTGCCCCG AAGT	924

5 coding for the protein having the amino-acid sequence SEQ ID NO:1

and SEQ ID NO:4

(mouse)

10 ATACCATTGT GCTGGAAAGG TTGCTGTGCC CGTCGCGTCG GGATTGTAAC TGTAATGGGC	60
ACCGAGGGGA AAGCCGGGAG GAAACTGTTG TTTCTCTCA CGTCTATGAT CCTGGGCTCT	120
TTGGTACAAG GCAAGGGTTC GGTGTACACT GCTCAATCTG ACGTCCAGGT TCCCGAGAAC	180
15 GAGTCCATCA AATTGACCTG CACCTACTCT GGCTTCTCCT CTCCCCGAGT GGAGTGGAAAG	240
TTCGTCCAAG GCAGCACAAAC TGCACTTGTG TGTATAACA GCCAGATCAC AGCTCCCTAT	300
20 GCGGACCGAG TCACCTTCTC ATCCAGTGGC ATCACGTTCA GTTCTGTGAC CCGGAAGGAC	360
AATGGAGAGT ATACTTGCAT GGTCTCCGAG GAAGGTGGCC AGAACTACGG GGAGGTCAGC	420
ATCCACCTCA CTGTGCTTGT ACCTCCATCC AAGCCGACGA TCAGTGTCCC CTCCCTGTC	480
25 ACCATTGGGA ACAGGGCAGT GCTGACCTGC TCAGAGCATG ATGGTTCCCC ACCCTCTGAA	540
TATTCCTGGT TCAAGGACGG GATATCCATG CTTACAGCAG ATGCCAAGAA AACCCGGGCC	600
30 TTCATGAATT CTTCATTCA CATTGATCCA AAGTCGGGGG ATCTGATCTT TGACCCCGTG	660
ACAGCCTTG ATAGTGGTGA ATACTACTGC CAGGCCAGA ATGGATATGG GACAGCCATG	720
AGGTCAGAGG CTGCACACAT GGATGCTGTG GAGCTGAATG TGGGGGGCAT CGTGGCAGC	780
35 GTCCTGGTAA CACTGATTCT CCTTGGACTC TTGATTTTG GCGTCTGGTT TGCCTATAGC	840
CGTGGATACT TTGAAACAAAC AAAGAAAGGG ACTGCACCGG GTAAGAAGGT CATTACAGC	900
40 CAGCCCAGTA CTCGAAGTGA GGGGGAATTCA AACAGACACT CGTCGTTCC GTGTCGACCT	960
GCTGCGGCTC CTCCGGTGTG CATTGGCTT ACTCAGGTGC TACAGGTTCC AGCCCTGCT	1020
GCTGTAGCTG CACAGGATGC CTTCAATGTC TTCTAGGTCC CACAGGACCC CTTGCTTTA	1080
45 TTCTAGCTAG GATATAAATT TAAAAACATC ATCTACTTCC CCCTCCTCTT TCCCACCCCTC	1140
CCTCCTTCC TTACCACCAT TGGGTGGCCC GAGACTAATT ACAAAAGTTT CGTTCCCCAT	1200
50 TCCTATGTGG GATTGGCAA GAGTCCTAGA CTAGACAGTA ATAGTGGCTG GGCTGACAGG	1260
AACCCAAACC AATACCTGGC TGTAAAGGCC TCTGAATAAG GACTTTAAGC CTAGCTCCCT	1320
GCTTCTCCT CCCCGGATGG GGTGCCAGCT ACTCTAGAAG GGGAGCTGCA TAAA	1374

55 coding for the protein having the amino-acid sequence SEQ ID NO:2.

The cDNA molecules will find their application in gene therapy, and they can be used as an oncosuppressor by transfection in carcinoma cells lacking this molecule.

- 5 The third aspect of the invention is directed to a gene coding for a protein of the present invention or a peptide derived from the protein. The gene will be used in the production of a protein or peptide of the invention. The flanking regions, such as promoter or leader sequences, are preferably chosen with regard to the expression system to be used to promote good production. Further, the codons
- 10 used in the gene may be selected with regard to the codons most frequently used by the selected expression host, in order to optimize the expression yield. For instance, if yeast is selected as the expression host, the codons may be optimized for yeast. The specific examples of genes of the invention are the protein coding regions of the exemplified cDNAs of the invention, namely the gene having the
- 15 partial nucleotide sequence

SEQ ID NO: 5

(part of human SEQ ID NO: 3)

ATGGGGACAA AGGCGCAAGT CGAGAGGAAA CTGTTGTGCC TCTTCATATT GGCGATCCTG	60
20 TTGTGCTCCC TGGCATTGGG CAGTGTACA GTGCACTCTT CTGAACCTGA AGTCAGAATT	120
CCTGAGAATA ATCCTGTGAA GTTGTCTGT GCCTACTCGG GCTTTCTTC TCCCCGTGTG	180
25 GAGTGGAAAGT TTGACCAAGG AGACACCACC AGACTCGTT GCTATAATAA CAAGATCACA	240
GCTTCCTATG AGGACCGGGT GACCTTCTTG CCAACTGGTA TCACCTCAA GTCCGTGACA	300
CGGGAAGACA CTGGGACATA CACTTGTATG GTCTCTGAGG AAGGCAGCAA CAGCTATGGG	360
30 GAGGTCAAGG TCAAGCTCAT CGTGCTTGTG CCTCCATCCA AGCCTACAGT TAACATCCCT	420
CCATCCAAGC CTACAGTTAA CATCCCCTCC TCTGCCACCA TTGGGAACCG GGCAGTGCTG	480
35 ACATGCTCAG AACAAAGATGG TTCCCCACCT TCTGAATACA CCTGGTTCAA AGATGGGATA	540
GTGATGCCTA CGAATCCCAA AAGCACCCGT TGCCTTCAGC AACTCTTCCT ATCTAGTCTG	600
AATCCCACAA CAGGAGAGCT GGTCTTTGAT CCCCTGTCA GCTCTGATAC TGGAGAATAC	660
40 AGCTGTGAGG CACGGAATGG GTATGGGACA CCCATGACTT CAAATCGTGT CGCGATGGAA	720
GCTGTGGACG GGAATGTGGG GGTCACTCGTG GCAGCCGTCC TTGTAACCT GATTCTCCTG	780
45 GGAATCTTGG TTTTGGCAT CTGGTTCCG TATAGCCGAG GCCACTTGA CAGAACAAAG	840
AAAGGGACTT CGAGTAAGAA GGTAGTTAC AGCCAGCCTA GTGCCCGAAG T	891

coding for the protein having the amino-acid sequence SEQ ID NO:1 and SEQ ID NO:6

(part of mouse SEQ ID NO:4)

	ATGGGCACCG AGGGAAAGC CGGGAGGAAA CTGTTGTTTC TCTTCACGTC TATGATCCTG	60
5	GGCTCTTGG TACAAGGCAA GGGTCGGTG TACACTGCTC AATCTGACGT CCAGGTTCCC	120
	GAGAACGAGT CCATCAAATT GACCTGCACC TACTCTGGCT TCTCCTCTCC CCGAGTGGAG	180
	TGGAAGTTCG TCCAAGGCAG CACAAGTGCA CTTGTGTGTT ATAACAGCCA GATCACAGCT	240
10	CCCTATGCGG ACCGAGTCAC CTTCTCATCC AGTGGCATCA CGTTCAGTTC TGTGACCCGG	300
	AAGGACAATG GAGAGTATACTTGCATGGTC TCCGAGGAAG GTGCCAGAA CTACGGGAG	360
15	GTCAGCATCC ACCTCACTGT GCTTGTACCT CCATCCAAGC CGACGATCAG TGTCCCCCTCC	420
	TCTGTACCA TTGGGAACAG GGCACTGCTG ACCTGCTCAG AGCATGATGG TTCCCCACCC	480
	TCTGAATATT CCTGGTTCAA GGACGGGATA TCCATGCTTA CAGCAGATGC CAAGAAAACC	540
20	CGGGCCTTCA TGAATTCTTC ATTCACCATT GATCCTAAAGT CGGGGGATCT GATCTTGAC	600
	CCCGTGACAG CCTTGATAG TGGTGAATAC TACTGCCAGG CCCAGAATGG ATATGGGACA	660
25	GCCATGAGGT CAGAGGCTGC ACACATGGAT GCTGTGGAGC TGAATGTGGG GGGCATCGTG	720
	GCAGCTGTCC TGGTAACACT GATTCTCCTT GGACTCTTGA TTTTGGCGT CTGGTTGCC	780
	TATAGCCGTG GATACTTTGA AACAACAAAG AAAGGGACTG CACCGGGTAA GAAGGTCATT	840
30	TACAGCCAGC CCAGTACTCG AAGTGAGGGG GAATTCAAAC AGACCTCGTC GTTCCGGTG	900

coding for the protein having the amino-acid sequence SEQ ID NO:2.

The proteins of the present invention can be chemically synthesized using standard methods known in the art, preferably solid state methods, such as the methods of Merrifield (J. Am. Chem. Soc. 85, 2149-2154 [1963]). Alternatively, the proteins of the present invention can be produced using methods of DNA recombinant technology (Sambrook et al. in "Molecular Cloning - A Laboratory Manual", 2nd. ed., Cold Spring Harbor Laboratory [1989]). Thus the fourth aspect of the invention is directed to a recombinant protein or peptide expressed by a structural gene or a fragment of the gene according to the present invention.

Preferably, DNA coding for a protein of the present invention is isolated through expression cloning. A cDNA expression library is constructed from a murine brain EC line (bEnd.3) as previously described (Fawcett et al., Nature 360, 481 [1992]). COS cells are transiently transfected with the cDNA library, stained in suspension with anti JAM antibody and then panned on plastic dishes coated with the appropriate second antibody.

- 10 -

A DNA sequence coding for a protein of the present invention is incorporated into a suitable expression vector which produces the requisite expression signals.

Expression vectors suitable for use in prokaryotic host cells are mentioned, for

5 example, in the aforementioned textbook of Maniatis et al. Such prokaryotic expression vectors which contain the DNA sequences coding for the proteins of the present invention operatively linked with an expression control sequence can be incorporated using conventional methods into any suitable prokaryotic cell. The selection of a suitable prokaryotic cell is determined by different factors which are
10 well-known in the art. Thus, for example, compatibility with the chosen vector, toxicity of the expression product, expression characteristics, necessary biological safety precautions and costs play a role and a compromise between all of these factors must be found.

Suitable prokaryotic organisms include gram-negative and gram-positive bacteria,

15 for example, E. coli and B. subtilis strains. Examples of prokaryotic organisms are E. coli strain M15, described as strain OZ 291 by Villarejo et al. in J. Bacteriol. 120, 466-474 (1974) and E. coli W3110 (ATCC No. 27325). In addition to the aforementioned E. coli strains, however, other generally accessible E. coli strains such as E. coli 294 (ATCC No. 31446) and E. coli RR1 (ATCC No. 31343) can also be used.

20 Expression vectors suitable for use in mammalian cells include but are not limited to pBC12MI [ATCC 67109], pSV2dhfr [ATCC 37146], pSVL [Pharmacia, Uppsala, Sweden], pRSVcat [ATCC 37152] and pMSG [Pharmacia, Uppsala]. A preferred vector for the expression of the proteins of the present invention is pECE.

25 Mammalian host cells that could be used include, e.g., human HeLa, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, CV1 African green monkey kidney cells, quail QC1-3 cells, Chinese hamster ovary (CHO) cells, mouse L cells and the COS cell lines. The CHO cell line (ATCC CCL 61) is preferred.

30 The manner in which the expression of the proteins of the present invention is carried out depends on the chosen expression vector/host cell system.

35 Usually, the prokaryotic host organisms which contain a desired expression vector are grown under conditions which are optimal for the growth of the prokaryotic host

organisms. At the end of the exponential growth, when the increase in cell number per unit time decreases, the expression of the desired protein is induced. The induction can be carried out by adding an inducer or a derepressor to the growth medium or by altering a physical parameter.

5

The mammalian host cells which contain a desired expression vector are grown under conditions which are optimal for the growth of the mammalian host cells. A typical expression vector contains the promoter element, which mediates the transcription of mRNA, the protein coding sequence, and the signals required for 10 efficient termination and polyadenylation of the transcript. Additional elements may include enhancers and intervening sequences bounded by spliced donor and acceptor sites.

Most of the vectors used for the transient expression of a given coding sequence 15 carry the SV40 origin of replication, which allows them to replicate to high copy numbers in cells (e.g. COS cells) that constitutively express the T antigen required to initiate viral DNA synthesis. Transient expression is not limited to COS cells. Any mammalian cell line that can be transfected can be utilized for this purpose. Elements that control a high efficient transcription include the early or the late 20 promoters from SV40 and the long terminal repeats (LTRs) from retroviruses, e.g. RSV, HIV, HTLV. However, also cellular signals can be used (e.g. human- β -actin-promoter).

Alternatively, stable cell lines carrying a gene of interest integrated into the 25 chromosome can be selected upon co-transfection with a selectable marker such as gpt, dhfr, neomycin or hygromycin.

Now, the transfected gene can be amplified to express large quantities of a foreign protein. The dihydrofolate reductase (DHFR) is a useful marker to develop lines of 30 cells carrying more than 1000 copies of the gene of interest. The mammalian cells are grown in increasing amounts of methotrexate. Subsequently, when the methotrexate is withdrawn, cell lines contain the amplified gene integrated into the chromosome.

35 The baculovirus-insect cell vector system can also be used for the production of the proteins of the present invention (for review see Luclow and Summers, Bio/Techno-

logy 6, 47-55 [1988]). The proteins produced in insect cells infected with recombinant baculovirus can undergo post-translational processing including N-glycosylation (Smith et al., Proc. Nat. Acad. Sci. USA 82, 8404-8408) and O-glycosylation (Thomsen et al., 12. International Herpesvirus Workshop, University of Philadelphia, Pennsylvania).

The proteins of the present invention can be purified from the cell mass or the culture supernatants according to methods of protein chemistry which are known in the art such as, for example, precipitation, e.g., with ammonium sulfate, dialysis, 10 ultrafiltration, gel filtration, ion-exchange chromatography, SDS-PAGE, isoelectric focusing, affinity chromatography like immunoaffinity chromatography, HPLC on normal or reverse systems or the like.

The fifth aspect of the invention is directed to an antibody binding specifically to a 15 protein according to the present invention or a part of the protein. The antibody may be polyclonal or monoclonal. In the experimental part of this specification the preparation of monoclonal antibodies of the invention is disclosed. One of the monoclonal antibodies of the invention mAb BV 12 binds specifically to JAM but does not inhibit transmigration of leukocytes through tight junctions, whereas 20 another monoclonal antibody of the invention mAb BV 11 not only binds specifically to JAM but also inhibits the transmigration of leukocytes through tight junctions. Both types of antibodies binding specifically to JAM may be used in diagnostics, and in diagnostic kits, e.g., for screening or detection of cell damage, particularly by detection of circulating JAM as a marker of early endothelial cell damage.

25 The sixth aspect of the invention is directed to a modifier of the polymerization of a transmembrane protein according to the present invention.

The term "modifier" is to be interpreted broadly and to comprise in the present 30 specification and appended claims, both inhibitors and activators of the polymerization of the JAM protein of the invention. Thus, the modifiers of the invention will either prevent or promote polymerization of JAM molecules at tight junctions, i.e., the dimerization, oligomerization or polymerization of JAM, or dedimerization, deoligomerization or depolymerization of JAM, respectively, at tight 35 junctions.

The modifier of the invention may be any ligand to the protein of the invention which binds to the protein and has the ability to prevent or promote the polymerization of the protein (JAM). For example, the modifier of the invention may have a structure which is complementary to the protein of the invention or a part of the protein.

- 5 However, in a preferred embodiment of this aspect of the invention the modifier is selected from the group consisting of polyclonal and monoclonal antibodies specifically binding to the protein according to the invention and inhibiting or inducing the polymerization of said protein, and polymerization-inhibiting or -inducing proteins, peptides, peptidomimetics and organic molecule-ligands
- 10 derived from the amino-acid sequence of the protein according to the invention.

The polyclonal and monoclonal antibodies of the invention can be produced by methods known in the art. These methods include the immunological method described by Kohler and Milstein in Nature 256, 495-497 (1975) and Campbell in "Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas" in Burdon et al., Eds., Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985) as well as by the recombinant DNA method described by Huse et al. in Science 246, 1275-1281 (1989).

20 The antibodies may be prepared in any mammal, including mice, rats, rabbits, goats and humans. The antibody may be a member of one of the following immunoglobulin classes; IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof, and preferably is an IgG antibody.

25 The seventh aspect of the invention is directed to a diagnostic kit comprising as a diagnostic reagent an antibody according to the invention or a modifier according to the invention. The actual diagnostic method which is going to be used will determine possible additional components in the kit, and the kit will preferably be

30 accompanied by instructions for use. An example of a widely used immunological diagnostic method is enzyme linked immunosorbent assay (ELISA), and this has been used in the experimental part of this specification.

35 The eight aspect of the invention is directed to a useful application of the modifier of the invention, namely a vaccine adjuvant comprising a modifier according to the present invention.

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The ninth aspect of the invention is directed to another useful and desirable application of the modifier of the invention, namely a medicament comprising as an active ingredient a modifier according to the present invention.

5

Examples of various applications of the modifiers of the invention are

- leukocyte infiltration
- tumor cell metastasis
- angiogenesis

10

- endothelial permeability
- detection of early endothelial cell damage
- adjuvants of oral vaccines, and
- making gut junctions more permeable to antigens, thus indicating use as a
- medicament for the therapeutic or prophylactic treatment of

15

acute and chronic inflammatory diseases, organ transplantation, myocardial ischemia, atherosclerosis, cancer, diabetic retinopathy, psoriasis, rheumatoid arthritis, intestinal infection.

20 The tenth aspect of the invention is directed to transgenic animals or cells overexpressing or lacking a protein according to the present invention.

Transgenic animals carrying null mutation of JAM created by standard techniques [Hogan et. al., Manipulating the mouse embryo: A laboratory manual. Cold Spring Harbor Laboratory Press N.Y. (1994)] will be used as *in vivo* models for screening 25 replacing, activating molecules for JAM and for providing the therapeutic potential of JAM in genetherapy in medicine.

JAM-overexpressing animals (e.g. using promoters selected from NSE, Thy 1, PDGFB, VE cadherin, Willebrand factor, and transomodulin) will be used for 30 screening *in vivo* for the therapeutic use of modifiers of JAM polymerization in medicine.

Transgenic cells are used for *in vitro* testing purposes.

35 Having now generally described this invention, the same will become better understood by reference to the specific examples, which are included herein for

- 15 -

purpose of illustration only and are not intended to be limiting unless otherwise specified.

Example 1

5

Generation of mAbs with JAM-neutralizing activity

Antibodies binding specifically to JAM were produced in the laboratory by standard hybridoma techniques as described in Martin-Padura et al., J. Biol. Chem. 269, 6124-
10 6132 (1994). Briefly, Lewis rat was immunized with a murine endothelial cell line
(H5V) [Garlanda et al., Proc. Natl. Acad. Sci. USA 91,7291-7295 (1994)]. Hybridomas
were produced by fusion of immunized rat splenocytes with Sp2/0 cell line from
ATCC (Maryland, USA). Hybridoma supernatants were screened by standard
15 enzyme-linked immunoassay (ELISA) for binding to H5V. Positive hybridoma were
then characterized by their ability to stain endothelial cell-cell contacts by
immunofluorescence microscopy which technique is disclosed below.

In order to select neutralizing mAbs, mAbs were screened on transmigration of
leukocytes through the endothelial monolayers (see below). Mabs BV11 and BV12
20 were selected after the first screening, and the corresponding hybridomas were
serially cloned twice by the method of limiting dilutions. MAbs isotypes were
determined using a rat isotyping kit (Sigma). Ascites were produced by a standard
technique [Martin-Padura et al., supra]. Briefly, Nu/Nu (CD1) BR mice were primed
with intraperitoneal injections of 0.5 ml of pristane 6 days before intraperitoneal
25 injection of 10X10⁶ hybridoma cells. Ascites were collected after 2-3 weeks. MAb
BV11 was purified from ascites by binding to immobilized protein G (Pharmacia), as
described by Martin -Padura et al., supra.

Example 2

30

Localization of JAM at tight junctions

Immunofluorescence microscopy

Cells were seeded on glass coverslips and grown to confluence in Medium 199
35 containing 20 % newborn calf serum before immunofluorescence staining. For some
cell types, glass coverslips were coated with human plasma fibronectin (7µg /ml).

Cells were fixed with MeOH for 4 min and processed for indirect immunofluorescence microscopy as previously described in detail by Lampugnani et al., J. Cell. Biol. 118, 1511-1522 (1992). Briefly, incubation with the primary antibody (mAb BV11 or mAb BV 12) was followed by rhodamine-conjugated secondary antibody (Dakopatts) in the presence of fluorescein-labelled phalloidin (1 μ g/ml) with several washes with 0.1% BSA in PBS between the various steps. Coverslips were then mounted in Mowiol 4-88 (Calbiochem). A Zeiss Axiophot microscope was used for observation and image recording on Kodak TMax P3200 films.

- 5 Confocal laser-scanning immunofluoresce microscopy was done on a Zeiss LSM 410 UV (Carl Zeiss). For simultaneous double-label fluorescence, an Argon ion laser operating at 488 nm and a Helium-Neon laser operating at 543 nm were used together with a band-pass filter combination of 510-525 and 590-610 for visualization of FICT and rhodamine fluorescence, respectively. RGB images were taken in high resolution mode using 1.024 X 1.024 image points (pixels) and 2s scan times. Regularly noise levels were reduced by several line averagings of the scans. Projection images were created from 0.8 μ m optical sections of tissue preparation or cell layers. To verify the distribution of JAM and cingulin, the individual images were separated electronically by a 20 pixel off-set along the abscissa.
- 10 JAM distributes selectively at cell-cell contacts in endothelial and epithelial cells. Distribution corresponds to other molecules located at tight junctions such as ZO-1 or cingulin. At confocal electron microscopy JAM localizes at tight junction while it is not found in other regions of cell-cell contacts such as adherence junctions.
- 15 JAM inhibits leukocyte transmigration
- 20 **A. In vitro assay in transwell units**
- 25 2x10⁵ endothelial cells were seeded on human plasma fibronectin (7 μ g/ml, Sigma) precoated polycarbonate membrane of Transwell units (24 mm diameter; 8.0 μ m pore size) and cultured for 4 to 5 days to confluence. Cultured medium from both upper and lower chambers were replaced with fresh medium and in some experiments mAbs were added to endothelial cell monolayers for 30 min and then allowed to stay during the transmigration assay. Monocytes were obtained from the

Example 3

JAM inhibits leukocyte transmigration

- 30 **A. In vitro assay in transwell units**
- 35 2x10⁵ endothelial cells were seeded on human plasma fibronectin (7 μ g/ml, Sigma) precoated polycarbonate membrane of Transwell units (24 mm diameter; 8.0 μ m pore size) and cultured for 4 to 5 days to confluence. Cultured medium from both upper and lower chambers were replaced with fresh medium and in some experiments mAbs were added to endothelial cell monolayers for 30 min and then allowed to stay during the transmigration assay. Monocytes were obtained from the

- peripheral blood of normal healthy donors as described by Colotta et al., J. Immunol. 132, 936 (1984). Briefly, monocytes (approximately 92% pure) were obtained from Ficoll-Hypaque separated mononuclear cells by centrifugation on a discontinuous (46%) gradient of isosmotic (285 mOsmol) Percoll (Pharmacia).
- 5 Polymorphonuclear cells were isolated by dextran sedimentation followed by Lymphoprep gradient and hypotonic lysis of erythrocytes, as previously described by Del Maschio et al., Br. J. Haematol. 72, 329-335 (1989). Cells to be used in suspension were resuspended at 10^7 cells/ml in complete medium and labeled by incubation with 100 mCi ^{51}Cr for 1h at room temperature. Cells were then washed
- 10 extensively and resuspended at 1.8×10^6 cells/ml in complete medium. An aliquot (1.5 ml) of radiolabelled cells was then added to each well and incubated for 60 min at 37°C. Non-adherent cells were then removed by washing gently with PBS plus 2% FCS from the upper chamber (non-adherent fraction). Transmigrated cells were collected from the lower chamber medium and removed by scraping with cotton
- 15 buds on the opposite face of the filter. These two fractions were pooled (migrated fraction). The intact EC monolayer together with the adherent leukocytes were collected by cutting the polycarbonate membrane (adherent fraction). Radioactivity of the three fractions was measured in a counter (Beckman).
- 20 The results are presented in Tables 1, 2 and 3 below.

Table 1. Spontaneous monocyte transmigration *in vitro* through an endothelial monolayer in the presence of different monoclonal antibodies.

	Treatment	cell number
30	Control	437713 ± 14351
	mAb BV11	190346 ± 23926**
	Irrelevant IgG2b	403027 ± 16214
	mAb BV12	381903 ± 9213
	mAb to CD31	415150 ± 12213

mAb BV11 and mAb BV12 are both antibodies of the invention.

mAb to CD31 is disclosed by Vecchi, A. et. al., Eur. J. Cell Biol. 63, 247-254 (1994)

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Antibodies were used as hybridoma supernatants at 1:2 dilution added to the upper chamber. Values are means \pm SEM of four experiments. ** p< 0.01 by analysis of various and Duncan's test. mAb BV11: monoclonal antibody binding to and neutralizing JAM; mAb BV12: monoclonal antibody binding to but not neutralizing JAM.

5

Table 2. Effect of mAb BV11 on MCP-1-induced monocyte transmigration through an endothelial monolayer.

10	Treatment	none	MCP-1
15	Control	323000 \pm 38400	825767 \pm 14351
	mAb BV11	172833 \pm 38933**	488967 \pm 13926**
	Irrelevant IgG2b	366100 \pm 38400	852250 \pm 16214

15

MCP-1 at 100 ng/ml was added to the lower compartment 5 min before monocyte seeding. Antibodies were used as hybridoma supernatants at 1:2 dilution added to the upper chamber. Values are means \pm SEM of two experiments. ** p< 0.01 by

20 analysis of various and Duncan's test. mAb BV11: monoclonal antibody neutralizing JAM.

25

Table 3. Effect of mAb BV11 on polymorphonuclear cell (PMN) migration through an endothelial monolayer.

25	Treatment	none	fMLP (10 nM)
30	Control	25566 \pm 3486	69400 \pm 2275
	mAb BV11	24852 \pm 3854	24852 \pm 1916**
	Irrelevant IgG2b	26890 \pm 3890	68890 \pm 1934

35

Chemotaxis was induced by addition of fMLP (500 nM) to the lower compartment of the Transwell unit. Antibodies were used as hybridoma supernatants at 1:2 dilution added to the upper chamber. Values are means \pm SEM of two experiments. ** p<

35

0.01 by analysis of various and Duncan's test. mAb BV11: monoclonal antibody neutralizing JAM.

- In conclusion, as reported in Tables 1, 2 and 3, addition of monoclonal antibody
 5 BV11 specifically binding to JAM resulted in the inhibition of both monocyte and polymorphonuclear cell transmigration. The antibody did not significantly alter the number of cells which remained adherent to the filter.

**B. *in vivo* assay: measurement of leukocyte recruitment in the air
 10 pouch model**

Mice were anesthetized with ether and 5 ml of sterile air were injected under the skin in the back (day 0). After three days pouches were reinjected with 3 ml of sterile air. On day 4, animals received intravenous injection of 200 µg of monoclonal antibody BV11 binding specifically to JAM or the same dose of
 15 nonimmune rat IgG (Sigma). On day 6; 1 ml of 1% carrageenan in saline was injected into the pouch. At different times after carrageenan the animals were anesthetized and the pouches were washed with 1 ml of saline. The lavage fluid was immediately cooled on ice and the volume was recorded. Then 50 µl were used for cell count after staining with erythrosin.

20

The results are presented in Table 4 below.

Table 4. Effect of mAb BV11 on neutrophil recruitment *in vivo*

25	Treatment	cell number ($\times 10^6$)
	Control	5.769 ± 0.932
	mAb BV11	3.629 ± 0.217*

30

Leukocyte recruitment was induced by injection of carrageenan in sterile saline (1 ml) into six day-old pouches. 200 µg of purified mAb BV11 (mAb binding to JAM) or rat non-immune-IgG (control) were injected intravenously in 200 µl 12 hours before carrageenan treatment. Animals were killed 48 hours after the treatment. Data are
 35 mean ± SD of at least seven animals in two experiments. * p< 0.002 according to Student's t test.

- 20 -

In conclusion, as reported in Table 4, the number of polymorphonuclear cells found in the air pouch after carrageenan injection was significantly reduced in the mice treated with the monoclonal antibody BV11 specifically binding to JAM in respect to mice IgG.

Table 5. Effect of mAb BV11 on paracellular permeability *in vivo*

Treatment	Control	mAb BV11
Exudate (ml)	1.49 ± 0.125	1.28 ± 0.217*

Measurement of permeability in the air pouch model was evaluated as collected exudate volume. Plasma exudation was induced by carrageenan in sterile saline (1 ml) in six day-old pouches. 200 µg of purified mAb BV11 (mAb binding to JAM) or rat non-immune-IgG (control) were injected intravenously in 200 µl 12 hours before carrageenan treatment. Animals were killed 48 hours after the treatment. Data are mean ± SD of at least 8 animals in two experiments. * p< 0.049 according to Student's t test.

Example 4

Inhibition of paracellular permeability by JAM

A. Constructs and Transfection
Constructs preparation and transfection procedures were performed according to Breviario et al., Arterioscler. Thromb. Vasc. Biol. 15, 1229-1239 (1995). JAM cDNA was isolated through expression cloning. The cDNA expression library was constructed from a murine brain EC line (bEnd.3) as previously described (Fawcett et al., Nature 360, 481 [1992]; Seed, Nature 329, 840 [1987]; Seed and Aruffo, Proc. Natl. Acad. Sci. U.S.A. 84, 3365 [1987]; Simmons et al. Nature 331, 624 [1988]). The cDNA library was oligo-dT primed bEnd.3 polyA + RNA cloned into pCDM8 (Nature 329, 840-842 [1987]). Plasmid pCDM8 was cut with HindIII and NotI enzymes and the insert was blunted and subcloned into the SmaI restriction site of pECE eucaryotic expression vector (Rutter et al., Cell 45, 721-732 [1986]) to give the pECE-JAM

construct. The construct was then checked for correct orientation by sequence analysis using the dideoxynucleotide chain termination method ("Molecular Cloning", Second Edition, Cold Spring Harbor Laboratory Press (1987) and Ausubel et al. (Eds) "Current Protocols in Molecular Biology", "Green Publishing Associates / Wiley-Interscience, New York (1990)). CHO cells were plated at $3\text{-}4 \times 10^6$ cells per 100 mm petri dish in DMEM with 10% FCS. 24 h after seeding cells were transfected by calcium phosphate precipitation method with 20 µg of pECE-JAM and 2 µg of plasmid pSV2 neo (Rutter et al., supra). After 24 h, the DNA-containing medium was replaced by fresh DMEM with 10% FCS and maintained for further 48 h. Then cells were detached and plated at 1×10^6 per 100 mm petri dish and cultured in selective medium with 600 µg/ml G418 (Geneticin, GIBCO). Resistant colonies were isolated and tested for BV11 antigen expression by immunofluorescence staining and immunoprecipitation analysis. Positive cells were cloned by limiting dilution and expanded for further studies.

B. Measurement of dextran passage in Transwell units
 Procedure to measuring permeability across the cell monolayer in Transwell units is extensively described in Breviaro et al., supra. Briefly, JAM transfectant or endothelial cells were seeded at 1.5×10^4 per 6.5 mm in Transwell units (polycarbonate filter, 0.4 mm pore, Costar) and cultured to confluence for 5 days. Then, culture medium was replaced with serum-free medium and fluorescein isothiocyanate-dextran (1 mg/ml, Sigma) was added to the upper chamber. At different times, 100 µl from the lower compartment were withdrawn and assayed by fluorimeter (excitation wavelength set at 492 nm and emission at 520 nm).

The results are presented in Table 6.

Table 6. Effect of JAM transfection on paracellular permeability.

30	Transfектант cells	% Permeability
Control		100.0 ± 2.5
JAM		46.5 ± 2.0
35	JAM + EGTA 5mM	110.0 ± 4.4
	JAM + Cyt D	91.0 ± 6.5

- 22 -

Values are means \pm SEM of three independent experiments/ ** p< 0.01 by analysis of various and Duncan's test.

- 5 Transfectants were seeded on Transwell filters, dextran was added to the upper compartment and its passage to the lower compartment was evaluated at 2 hours. Permeability in JAM transfectants was increased by addition of EGTA and cytochalasin D indicating that the activity is Ca⁺⁺ dependent and requires an intact actin cytoskeleton.
- 10 In conclusion, as reported in Table 6, JAM transfection significantly reduced the passage of dextran through intercellular junctions. As reference the Table also reports the effect of permeability increasing agents such as EGTA and cytochalasin D. This also shows that JAM needs Ca⁺⁺ and an intact actin cytoskeleton to exert its effect.
- 15

Example 5

Use of mAb BV11 to detect JAM in ELISA assay

20 **ELISA sandwich**

- An ELISA standard protocol was followed [Peri et al., J. Immunol. Meth. 174, 249-257, (1994)]. Briefly, 96 well ELISA plates (Falcon) were coated with 50 μ l/well of rabbit anti-JAM serum diluted 1/3000 in 15 mM carbonate buffer, pH 9.6 and incubated overnight at 4°C. After incubation, plates were washed three times with PBS + 0.05% Tween 20 (washing buffer). Non-specific binding was blocked with 5% dry milk in washing buffer for 2h at room temperature . After wash, JAM-containing samples were added for 2h at 37°C. Then, plates were washed and incubated with mAb BV11 for 1h at 37°C. Peroxidase conjugated anti-rat IgG (diluted 1:2000, Sigma) was incubated for 1h at RT and then 100 μ l chromogen substrate was added.
- 25
- 30 Adsorbance values were read at 405 nm.

The results are presented in Table 7.

Table 7. Detection of JAM (O.D.) on different cells in ELISA

Treatment	endothelial cells	3T3 fibroblasts	MeI(hemopoietic line)
Control	150 ± 10	136 ± 21	166 ± 14
mAb BV 11	920 ± 7 **	142 ± 16	169 ± 19
non-immune serum	167 ± 5	148 ± 9	179 ± 9

10 Control: only secondary antibody was added. Rabbit anti-JAM serum or non-immune rabbit serum were used at dilution 1/3000. Adsorbance values are means ± SEM of four replicates/ ** p< 0.01 by analysis of various and Duncan's test.

15 In conclusion, in ELISA the mAb BV11 was able to detect JAM protein in solubilized endothelial cells and JAM transfectant cells while it gave negative values using the extracts of cells which do not express JAM such as hemopoietic precursor cell lines and 3T3 fibroblasts.

Example 6

20

Expression of soluble JAM

An extracellular JAM fragment was cloned by PCR [Saiki et al., Science 239, 487 (1988)] from full length JAM cDNA introducing a Kpn1 restriction site upstream the start codon and a stop codon at position 775 just in front a HindIII restriction site. The amplified DNA was cut by the restriction enzymes Kpn1 and HindIII and the resulting DNA was ligated into the cloning site of the baculovirus expression vector pFASTBAC1 (Gibco BRL). The vector was transformed into DH10Bac cells (Gibco BRL), the transformants were plated and stained with x-Gal according to the recommendation of the manufacturer. After selection of a recombinant vector, the DNA was transfected into Sf9 cells. Soluble JAM was detected in the cell culture medium seven days after transfection with the vector or three days after infection with the recombinant baculovirus.

35 Purified soluble JAM aggregates by itself depending on the solvent conditions. Inhibitors of the JAM self aggregation can be identified by physical methods (light

scattering, ultracentrifugation, gelpermeation chromatography, BiaCore etc.) or, as described below, by a two-sided sandwich type immunoassay using the monoclonal antibodies of this invention (mab BV11 and mab BV12). Briefly, Immunoplates (Nunc Maxisorb) are coated overnight with 100 µl/well of a solution of mab BV12 (10 µg/ml) in 0.1 M sodium bicarbonate buffer. The wells are blocked by addition of blocking buffer (1% bovine serum albumin in Tris-buffered saline, 0.05% Tween 20 pH 7.5; 100 µl/well). After three hours the wells are washed and the sample of soluble JAM is added together with the aggregation inhibitor at suitable dilution. After incubation overnight in the cold the sample is removed, the wells are washed and an antibody-enzyme conjugate is added at suitable concentration diluted with blocking buffer. The antibody-enzyme conjugate may be prepared by coupling mab BV12 to activated horseradish peroxidase according to Nakane and Kawaoi (J. Histochem. Cytochem. 22, 1084-1091 [1975]). The plate is washed and incubated with a colorimetric enzyme substrate, e.g., tetramethyl benzidine and hydrogenperoxide. Inhibitors of JAM self aggregation are recognized by reduction of the bound peroxidase activity. A similar assay using mab BV11 or BV12 for coating the plate can be used for measuring soluble JAM as diagnostic marker of murine endothelial cell damage / proliferation.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: Transmembrane component of tight junction

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: Apple Macintosh
- (C) OPERATING SYSTEM: System 7.1 (Macintosh)
- (D) SOFTWARE: Word 5.0

(vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: SE 960 4470-6
- (B) FILING DATE: 04-DEC-1996

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 298 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Met Gly Thr Lys Ala Gln Val Glu Arg Lys Leu Leu Cys Leu Phe Ile
1 5 10 15

Leu Ala Ile Leu Leu Cys Ser Leu Ala Leu Gly Ser Val Thr Val His
20 25 30

Ser Ser Glu Pro Glu Val Arg Ile Pro Glu Asn Asn Pro Val Lys Leu
35 40 45

- 26 -

Ser Cys Ala Tyr Ser Gly Phe Ser Ser Pro Arg Val Glu Trp Lys Phe
 50 55 60

5 Asp Gln Gly Asp Thr Thr Arg Leu Val Cys Tyr Asn Asn Lys Ile Thr
 65 70 75 80

Ala Ser Tyr Glu Asp Arg Val Thr Phe Leu Pro Thr Gly Ile Thr Phe
 10 85 90 95

Lys Ser Val Thr Arg Glu Asp Thr Gly Thr Tyr Thr Cys Met Val Ser
 100 105 110

15 Glu Glu Gly Gly Asn Ser Tyr Gly Glu Val Lys Val Lys Leu Ile Val
 115 120 125

Leu Val Pro Pro Ser Lys Pro Thr Val Asn Ile Pro Pro Ser Lys Pro
 130 135 140

20 Thr Val Asn Ile Pro Ser Ser Ala Thr Thr Ile Gly Asn Arg Ala Val
 145 150 155 160

Leu Thr Cys Ser Glu Gln Asp Gly Ser Pro Pro Ser Glu Tyr Thr Trp
 25 165 170 175

Phe Lys Asp Gly Ile Val Met Pro Thr Asn Pro Lys Ser Thr Arg Cys
 180 185 190

30 Leu Gln Gln Leu Phe Leu Ser Ser Leu Asn Pro Thr Thr Gly Glu Leu
 195 200 205

Val Phe Asp Pro Leu Ser Ala Ser Asp Thr Gly Glu Tyr Ser Cys Glu
 35 210 215 220

Ala Arg Asn Gly Tyr Gly Thr Pro Met Thr Ser Asn Arg Val Ala Met
 225 230 235 240

Glu Ala Val Asp Gly Asn Val Gly Val Ile Val Ala Ala Val Leu Val
 40 245 250 255

Thr Leu Ile Leu Leu Gly Ile Leu Val Phe Gly Ile Trp Phe Pro Tyr
 260 265 270

45 Ser Arg Gly His Phe Asp Arg Thr Lys Lys Gly Thr Ser Ser Lys Lys
 275 280 285

Val Val Tyr Ser Gln Pro Ser Ala Arg Ser
 50 290 295

(2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 300 amino acids

- 27 -

- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: both

5 (ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

10 (v) FRAGMENT TYPE: N-terminal

15 (ix) FEATURE:

- (A) NAME/KEY: Disulfide-bond
- (B) LOCATION:49..108

20 (ix) FEATURE:

- (A) NAME/KEY: Disulfide-bond
- (B) LOCATION:152..212

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Gly Thr Glu Gly Lys Ala Gly Arg Lys Leu Leu Phe Leu Phe Thr
1 5 10 15

30 Ser Met Ile Leu Gly Ser Leu Val Gln Gly Lys Gly Ser Val Tyr Thr
20 25 30

Ala Gln Ser Asp Val Gln Val Pro Glu Asn Glu Ser Ile Lys Leu Thr
35 40 45

35 Cys Thr Tyr Ser Gly Phe Ser Ser Pro Arg Val Glu Trp Lys Phe Val
50 55 60

40 Gln Gly Ser Thr Thr Ala Leu Val Cys Tyr Asn Ser Gln Ile Thr Ala
65 70 75 80

Pro Tyr Ala Asp Arg Val Thr Phe Ser Ser Ser Gly Ile Thr Phe Ser
85 90 95

45 Ser Val Thr Arg Lys Asp Asn Gly Glu Tyr Thr Cys Met Val Ser Glu
100 105 110

Glu Gly Gly Gln Asn Tyr Gly Glu Val Ser Ile His Leu Thr Val Leu
115 120 125

50 Val Pro Pro Ser Lys Pro Thr Ile Ser Val Pro Ser Ser Val Thr Ile
130 135 140

Gly Asn Arg Ala Val Leu Thr Cys Ser Glu His Asp Gly Ser Pro Pro
145 150 155 160

Ser Glu Tyr Ser Tri Phe Lys Asp Gly Ile Ser Met Leu Thr Ala Asp
165 170 175

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Ala Lys Lys Thr Arg Ala Phe Met Asn Ser Ser Phe Thr Ile Asp Pro
 180 185 190
 5 Lys Ser Gly Asp Leu Ile Phe Asp Pro Val Thr Ala Phe Asp Ser Gly
 195 200 205
 Glu Tyr Tyr Cys Gln Ala Gln Asn Gly Tyr Gly Thr Ala Met Arg Ser
 210 215 220
 10 Glu Ala Ala His Met Asp Ala Val Glu Leu Asn Val Gly Gly Ile Val
 225 230 235 240
 Ala Ala Val Leu Val Thr Leu Ile Leu Leu Gly Leu Leu Ile Phe Gly
 15 245 250 255
 Val Trp Phe Ala Tyr Ser Arg Gly Tyr Phe Glu Thr Thr Lys Lys Gly
 260 265 270
 20 Thr Ala Pro Gly Lys Lys Val Ile Tyr Ser Gln Pro Ser Thr Arg Ser
 275 280 285
 Glu Gly Glu Phe Lys Gln Thr Ser Ser Phe Leu Val
 290 295 300
 25

(2) INFORMATION FOR SEQ ID NO: 3:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 924 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

40 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

45 TCCATTGTGC TCTAAAGCGG GACGCTGATC GCGATGGGGA CAAAGGCGCA AGTCGAGAGG 60
 AAACTGTTGT GCCTCTTCAT ATTGGCGATC CTGTTGTGCT CCCTGGCATT GGGCAGTGT 120
 ACAGTGCACT CTTCTGAACC TGAAGTCAGA ATTCCTGAGA ATAATCCTGT GAAGTTGTCC 180
 50 TGTGCCTACT CGGGCTTTTC TTCTCCCCGT GTGGAGTGGA AGTTTGACCA AGGAGACACC 240
 ACCAGACTCG TTTGCTATAA TAACAAGATC ACAGCTTCCT ATGAGGACCG GGTGACCTTC 300
 55 TTGCCAACTG GTATCACCTT CAAGTCCGTG ACACGGGAAG ACACTGGGAC ATACACTTGT 360

ATGGTCTCTG AGGAAGGCGG CAACAGCTAT GGGGAGGTCA AGGTCAAGCT CATCGTGCTT 420
 GTGCCTCCAT CCAAGCCTAC AGTTAACATC CCTCCATCCA AGCCTACAGT TAACATCCCC 480
 5 TCCTCTGCCA CCATTGGAA CGGGCAGTG CTGACATGCT CAGAACAAAGA TGGTCCCCA 540
 CCTTCTGAAT ACACCTGGTT CAAAGATGGG ATAGTGATGC CTACGAATCC CAAAAGCACC 600
 10 CGTTGCCTTC AGCAACTCTT CCTATCTAGT CTGAATCCC CAACAGGAGA GCTGGTCTTT 660
 GATCCCCTGT CAGCCTCTGA TACTGGAGAA TACAGCTGTG AGGCACGGAA TGGGTATGGG 720
 ACACCCATGA CTTCAAATCG TGTCGCGATG GAAGCTGTGG ACGGAAATGT GGGGGTCATC 780
 15 GTGGCAGCCG TCCTTGTAAAC CCTGATTCTC CTGGGAATCT TGGTTTTGG CATCTGGTTT 840
 CCGTATAGCC GAGGCCACTT TGACAGAACAAAGAAAGGGAA CTTCGAGTAA GAAGGTAGTT 900
 20 TACAGCCAGC CTAGTGCCCG AAGT 924

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 1374 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ATACCATTGT GCTGGAAAGG TTGCTGTGCC CGTCGCGTCG GGATTGTAAAC TGTAATGGGC 60
 40 ACCGAGGGGA AAGCCGGGAG GAAACTGTIG TTTCTCTTCA CGTCTATGAT CCTGGGCTCT 120
 TTGGTACAAG GCAAGGGTTC GGTGTACACT GCTCAATCTG ACGTCCAGGT TCCCGAGAAC 180
 45 GAGTCCATCA AATTGACCTG CACCTACTCT GGCTTCTCCT CTCCCCGAGT GGAGTGGAAG 240
 TTCGTCCAAG GCAGCACAAAC TGCACTTGTG TGTATAACA GCCAGATCAC AGCTCCCTAT 300
 50 GCGGACCGAG TCACCTTCTC ATCCAGTGGC ATCACGTTCA GTTCTGTGAC CCGGAAGGAC 360
 AATGGAGAGT ATACTTGCAT GGTCTCCGAG GAAGGTGGCC AGAACTACGG GGAGGTCAGC 420
 ATCCACCTICA CTGTGCTTGT ACCTCCATCC AAGCCGACGA TCAGTGTCCC CTCCCTGTGTC 480
 55 ACCATTGGGA ACAGGGCAGT GCTGACCTGC TCAGAGCATG ATGGTTCCCC ACCCTCTGAA 540
 TATTCTGGT TCAAGGACGG GATATCCATG CTTACAGCAG ATGCCAAGAA AACCCGGGCC 600

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	TTCATGAATT CTTCATCAC CATTGATCCA AAGTCGGGGG ATCTGATCTT TGACCCCGTG	660
5	ACAGCCTTG ATAGTGGTGA ATACTACTGC CAGGCCAGA ATGGATATGG GACAGCCATG	720
	AGGTCAAGGG CTGCACACAT GGATGCTGTG GAGCTGAATG TGGGGGGCAT CGTGGCAGCT	780
	GTCCTGGTAA CACTGATTCT CCTTGGACTC TTGATTTTG GCGCTGGTT TGCCTATAGC	840
10	CGTGGATACT TTGAAACAAC AAAGAAAGGG ACTGCACCGG GTAAGAAGGT CATTACAGC	900
	CAGCCCAGTA CTCGAAGTGA GGGGAATTCA AACAGACCT CGTCGTTCC GGTGTGACCT	960
15	GCTGCGGCTC CTCCGTTGTC CATTGCCTT ACTCAGGTGC TACAGGTTCC AGCCCTGCT	1020
	GCTGTAGCTG CACAGGATGC CTTCAATGTC TTCTAGGTCC CACAGGACCC CTTGCTTTA	1080
	TTCTAGCTAG GATATAAATT TAAAAACATC ATCTACTTCC CCCTCCTCTT TCCCACCCCTC	1140
20	CCTCCTTCC TTACCACCAT TGGGTGGCCC GAGACTAATT ACAAAAGTTT CGTTCCCCAT	1200
	TCCTATGTGG GATTGGGCAA GAGTCCTAGA CTAGACAGTA ATAGTGGCTG GGCTGACAGG	1260
25	AACCCAAACC AATACTGGC TGTAAAGGCC TCTGAATAAG GACTTTAACG CTAGCTCCCT	1320
	GCTTTCCTT CCCCCGGATGG GGTGCCAGCT ACTCTAGAAG GGGAGCTGCA TAAA	1374

(2) INFORMATION FOR SEQ ID NO: 5:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 891 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA to mRNA

40 (iii) HYPOTHETICAL: NO

45 (iv) ANTI-SENSE: NO

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

45	ATGGGGACAA AGGCGCAAGT CGAGAGGAAA CTGTTGTGCC TCTTCATATT GGCGATCCTG	60
	TTGTGCTCCC TGGCATTGGG CAGTGTACA GTGCACTCTT CTGAACCTGA AGTCAGAATT	120
50	CCTGAGAATA ATCCTGTGAA GTTGTCTGT GCCTACTCGG GCTTTCTTC TCCCCGTGTG	180
	GAGTGGAAAGT TTGACCAAGG AGACACCAAGC AGACTCGTT GCTATAATAA CAAGATCACA	240
	GCTTCCTATG AGGACCGGGT GACCTTCTTG CCAACTGGTA TCACCTCAA GTCCGTGACA	300
55	CGGGAAAGACA CTGGGACATA CACTTGTATG GTCTCTGAGG AAGGCGGCAA CAGCTATGGG	360
	GAGGTCAAGG TCAAGCTCAT CGTGTGTTGTG CCTCCATCCA AGCCTACAGT TAACATCCCT	420

5 CCATCCAAGC CTACAGTTAA CATCCCCCTCC TCTGCCACCA TTGGGAACCG GGCAGTGCTG 480
 ACATGCTCAG ACAAAGATGG TTCCCCACCT TCTGAATACA CCTGGTTCAA AGATGGATA 540
 GTGATGCCTA CGAACATCCCAA AAGCACCCGT TGCCTTCAGC AACTCTTCCT ATCTAGTCTG 600
 AATCCCACAA CAGGAGAGCT GGTCTTGAT CCCCTGTAG CCTCTGATAC TGGAGAATAC 660
 10 AGCTGTGAGG CACGGAATGG GTATGGGACA CCCATGACTT CAAATCGTGT CGCGATGGAA 720
 GCTGTGGACG GGAATGTGGG GGTCACTCGTG GCAGCCGTCC TTGTAACCCT GATTCTCCTG 780
 15 GGAATCTTGG TTTTGGCAT CTGGTTCCG TATAGCCGAG GCCACTTGA CAGAACAAAG 840
 AAAGGGACTT CGAGTAAGAA GGTAGTTAC AGCCAGCCTA GTGCCCGAAG T 891

20 (2) INFORMATION FOR SEQ ID NO: 6:

- 25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 900 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- 30 (ii) MOLECULE TYPE: cDNA to mRNA
 (iii) HYPOTHETICAL: NO

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

40 ATGGGCACCG AGGGGAAAGC CGGGAGGAAA CTGTTGTTTC TCTTCACGTC TATGATCCTG 60
 GGCTCTTGG TACAAGGCAA GGGTCGGTG TACACTGCTC AATCTGACGT CCAGGTTCCC 120
 GAGAACGAGT CCATCAAATT GACCTGCACC TACTCTGGCT TCTCCTCTCC CCGAGTGGAG 180
 45 TGGAAAGTTCG TCCAAGGCAG CACAAGTGCA CTTGTGTGTT ATAACAGCCA GATCACAGCT 240
 CCCTATGCGG ACCGAGTCAC CTTCTCATCC AGTGGCATCA CGTTCAGTT TGTGACCCGG 300
 AAGGACAATG GAGAGTATAC TTGCATGGTC TCCGAGGAAG GTGCCAGAA CTACGGGGAG 360
 50 GTCAGCATCC ACCTCACTGT GCTTGTACCT CCATCCAAGC CGACGATCAG TGTCCCCCTCC 420
 TCTGTACCA TTGGGAACAG GGCAGTGCTG ACCTGCTCAG AGCATGATGG TTCCCCACCC 480
 55 TCTGAATATT CCTGGTTCAA GGACGGGATA TCCATGCTTA CAGCAGATGC CAAGAAAACC 540
 CGGGCCTTCA TGAATTCTTC ATTCAACCATT GATCCAAAGT CGGGGGATCT GATCTTGAC 600
 CCCGTGACAG CCTTGATAG TGGTGAATAC TACTGCCAGG CCCAGAATGG ATATGGGACA 660
 GCCATGAGGT CAGAGGCTGC ACACATGGAT GCTGTGGAGC TGAATGTGGG GGGCATCGTG 720

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GCAGCTGTCC TGGTAACACT GATTCTCCTT GGACTCTTGA TTTTTGGCGT CTGGTTGCC 780
TATAGCCGTG GATACTTGA AACAAACAAAG AAAGGGACTG CACCGGGTAA GAAGGTCATT 840
5 TACAGCCAGC CCAGTACTCG AAGTGAGGGG GAATTCAAAC AGACCTCGTC GTTCCTGGTG 900

CLAIMS

1. Protein in glycosylated or unglycosylated form comprising an amino-acid sequence selected from the sequence SEQ ID NO:1 and homologous sequences having at least 72% homology thereto.
5
2. Protein according to claim 1, wherein said sequence is SEQ ID NO:2
3. DNA sequence coding for a protein as claimed in claim 1 or 2.
10
4. DNA sequence according to claim 3 selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:4
5. Gene coding for a protein as claimed in claim 1 or a peptide derived therefrom.
15
6. Gene according to claim 5 selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:6.
7. Vector comprising a DNA sequence as claimed in claim 4 or a gene as claimed
20 in claim 5 or 6.
8. Organisms transformed with a vector as claimed in claim 7.
9. Recombinant protein or peptide expressed by a gene or a fragment of the gene
25 according to claim 5 or 6.
10. Antibody binding specifically to a protein according to claim 1 or a part of the protein.
- 30 11. Modifier of the polymerization of a transmembrane protein defined in claim 1.
12. Modifier according to claim 11 selected from the group consisting of antibodies specifically binding to the protein defined in claim 1 and inhibiting or inducing the polymerization of the protein, and polymerization-inhibiting or -inducing proteins,
35 peptides, peptidomimetics and organic molecule-ligands derived from the amino-acid sequence of the protein defined in claim 1.

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13. Diagnostic kit comprising as a diagnostic reagent an antibody according to claim 10 or a modifier according to claim 11 or 12.
- 5 14. Vaccine adjuvant comprising a modifier according to claim 11 or 12.
15. Medicament comprising as an active ingredient a modifier according to claim 11 or 12.
- 10 16. The use of a protein according to claim 1 for identifying and isolating modifiers of its polymerization.
17. The use of a modifier according to claim 11 or 12 for the preparation of medicaments.
- 15 18. A method for producing a protein as claimed in claim 1 or 2, comprising cultivating an organism as claimed in claim 8 in a suitable medium and optionally isolating said protein.
- 20 19. Proteins as claimed in claim 1 or 2 prepared by the method of claim 18.
20. A modifier according to claim 11 or 12 as active ingredient in medicaments.
21. The invention substantially as hereinbefore described, especially with
25 reference to the examples.

INTERNATIONAL SEARCH REPORT

Inte onal Application No

PCT/EP 97/06723

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12	C07K14/705	C07K16/28	G01N33/53	G01N33/566
A61K39/395	A61K38/17	A01K67/027		

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K G01N A61K A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NAIK U.P. ET AL.: "Mechanisms of platelet activation by a stimulatory antibody: cross linking of a novel platelet receptor for monoclonal antibody F11 with the Fc-gammaRII receptor." BIOCHEMICAL JOURNAL, vol. 310, 1995, pages 155-162, XP002060366 see table 2 ---	1-21
X	WANG F. ET AL.: "Stimulatory antibody-induced activation and selective translocation of protein kinase C isoenzymes in human platelets." BIOCHEMICAL JOURNAL, vol. 311, no. 2, 1995, pages 401-406, XP002060055 see the whole document ---	1-21
	-/-	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

26 March 1998

08/04/1998

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Authorized officer

Mandl, B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/06723

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT.		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>SOBOCKA M B ET AL: "Identification, characterization and distribution of a novel receptor protein (F11), recognized by a platelet-activating monoclonal antibody, in cells of the cardiovascular system."</p> <p>ABSTRACTS SUBMITTED TO THE 36TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF HEMATOLOGY, NASHVILLE, TENNESSEE, USA, DECEMBER 2-6, 1994. BLOOD 84 (10 SUPPL. 1). 1994. 473A. ISSN: 0006-4971, XP002060056 see abstract</p> <p>---</p>	1, 2, 10-17, 19-21
A	<p>FURUSE M. ET AL.: "Occludin: A novel integral membrane protein localizing at tight junctions."</p> <p>JOURNAL OF CELL BIOLOGY, vol. 123, no. 6, 1993, pages 1777-1788, XP002060057 cited in the application see the whole document</p> <p>---</p>	1-21
P, X	<p>US 5 665 701 A (THE RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK) 9 September 1997 see the whole document</p> <p>-----</p>	1-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. onal Application No

PCT/EP 97/06723

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5665701 A	09-09-97	NONE	

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